ISSN-O : 2349 9400; P : 0974 6315

Journal of Crop and Weed, 19(3):69-76 (2023)

https://www.cwss.in
www.cropandweed.com

Research Article

Bio-pigment extraction from three red colour tropical flowers using aqueous media

D. SARKAR, *A. MANDAL KHAN, I. SARKAR, S. MAITRA AND [1]P. K. PAUL

Department of Floriculture, Medicinal and Aromatic Plants
[1]Department of Pomology and Post-Harvest Technology
Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya,
Pundibari-736165, Cooch Behar, West Bengal, India

Received: 06.06.2023; Revised: 24.09.2023; Accepted: 08.12.2023

DOI: https://doi.org/10.22271/09746315.2023.v19.i3.1743

ABSTRACT

*Use of natural sources of colour is being emphasized worldwide and flowers are one of the most potential sources. The present study aims to evaluate the quality of pigments obtained from common red colour flowers Erythrina indica, Delonix regia and Spathodia campanulata, through different aqueous extraction methods. Visible differences in colour represented by l*a*b* and ΔE* values were noted among the eighteen pigments. Soaking and maceration in acidic solution resulted in pigments with unique colours in all the three tropical flower species. Microwave assisted extraction increases the pH of pigments (6.32) compared to cold water (5.90). Pigments of Spathodia showed temperature stability till 103.66°C. Anthocyanin and carotenoid content varied significantly among the flower species and with methods of extraction. Maximum anthocyanin was derived from S. campanulata through hot water extraction (80°C) while microwave assisted extraction resulted in higher anthocyanin extraction but lower carotenoid content compared to other extraction methods employed. These ornamental plants can be exploited as sources of bio-pigments.*

***Keywords*:** Anthocyanin, carotenoid, bio-pigment, colour difference and pigment extraction

Natural dyes were in vogue since the beginning of civilization for colouring food, fabric, leather and even human body and are re-gaining fast popularity worldwide owing to increasing health awareness. The global market for natural dyes and pigments is growing at 4.8% and likely to attain US$ 4.67 billion by 2023 (Rymbai *et al.*, 2011). Common synthetic dyes like cationic dyes are reported to cause tachycardia, shock, queasiness, jaundice, tetraplegia and haemolytic anaemia etc. (Vadivelan and Kumar, 2005). Azo dyes, the leading variety of synthetic dyes, are transformed into colourless scented amines, which have the potential to be poisonous and cancer-causing (Yaneva and Georgieva, 2012). On the contrary, the natural dyes are eco-friendly and non-toxic. Hence, the search and research for newer natural pigments has received impetus in recent days. Different plants or plant parts, minerals, insects and animals can be used to derive natural dyes (Verma and Gupta, 2017). Among botanical sources, berry, flower, bark, leaf and seed of specific plants are common. Flowers are the most common and diverse source of pigments in the nature. The flowers of ornamental trees can be exploited for natural dye extraction as these blooms convert into mere waste after aesthetic gratification. The Terai and Dooars region of West Bengal has an enviable floristic diversity, showcasing blooms in assorted colour shades. The present study aims at identification of potential red coloured dye yielding flowering tree species and also standardization of the pigment extraction process. Common sources for red colour in natural dyes are annatto (*Bixa orellana*), beetroot extract, flowers of *Spathodea campanulata* (Lokesh and Swamy, 2013), *Althea rosea* (Siva, 2007), *Pterocarpus santalinus* (Siva, 2007) etc.

**Email: meet.arpitakhan@gmail.com*

How to cite: Sarkar, D., Mandal Khan, A., Sarkar, I., Maitra, S. and Paul, P.K. 2023. Bio-pigment extraction from three red colour tropical flowers using aqueous media. *J. Crop and Weed*, 19(3): 69-76.

E. indica or Coral Tree flowers in leafless condition in the late winter. Among the 130 species of the genus a few like *E. stricta* (Devi, 2019), *E. crista-galli* (Susetyarini *et al*., 2020) and *E. suberosa* (Singh and Purohit, 2012) have been exploited for pigment extraction. *Delonix regia* is an evergreen plant that flowers during the summer months *i.e.* April to May. Bio-pigment from Delonix flowers was tested on silk and cotton fabrics (Vankar and Shanker, 2009; Divya and Manonmani, 2014). The large blooms of *S. campanulata* are cited throughout the year with peak flowerings during April to May and October to December. The species is distributed all over the country. Application of this flower pigment on silk and cotton was reported by Lokesh and Swamy, 2013; Singh, 2017; Parthasarathi and Lokesh, 2015; Patil *et al.*, 2016. In the Terai region of West Bengal, *E. indica*, *D. regia*, *Bombax ceiba*, *Beautea monosperma* and *S. campanulata* are predominant red flowering trees dominating the wastelands, roadsides and natural forests. In the present study, bio-pigments extracted from flowers of *E. indica*, *D. regia* and *S. campanulata* through different aqueous extraction methods were evaluated for their chemical content, colour values and stability.

MATERIALS AND METHODS

The experiment was carried out at the Uttar Banga Krishi Viswavidyalaya, West Bengal, during 2018 to 2020. Flowers of the three species, *E. indica* (S_1), *D. regia* (S_2) and *S. campanulata* (S_3) were collected from the road side plantations and were subjected to six methods of pigment extraction *viz.* soaking in cold water (18-20°C) and maceration (M_1), soaking in hot water (80°C) and maceration (M_2), boiling in water at 100°C (M_3), microwave assisted extraction (Whirlpool 50006, at 100% power level for 4 minutes) (M_4), soaking and maceration in acidic solution (pH 4.5-5) (M_5) and soaking and maceration in alkaline solution (pH 8.00) (M_6).

After collection of flowers, the petals were separated, rinsed with tap water, dried under shade and stored in air-tight containers for extraction of pigments. Flower parts, apart from petals, were considered as wastage and was recorded for each species in percent weight. The dried petals of each species were subjected to six methods of extraction. Two grams of petals was processed in 100ml of double distilled water/ solution in all the methods. In first and second methods, soaking was carried out in cold (18-20°C) and hot water (80°C), respectively for 1 hour followed by maceration in electric grinder for 5 minutes. For the third method (M_3), the petals were boiled in a water-bath until most of the pigment was extracted. For further analysis, the pigment extract's volume was increased to 100 ml. Microwave assisted extraction (M_4) was carried out in a microwave oven at high power for 5 minutes. For the fifth and sixth methods acidic solution (pH-4.5) and alkaline solution (pH-8.00) were prepared using citric acid (500ppm) and sodium hydroxide (500ppm), respectively. The petals were soaked for 15 minutes in the solutions and macerated in a grinder. After extraction, the extracts were first strained through a fine mesh strainer to separate out the petal mass followed by filtration through Whatman-1 filter paper to obtain clear pigment extracts which were subjected to further analysis.

The colour of fresh petals of the three species were recorded with RHS colour chart and Hunter Colour Meter. For each species, the fresh weight of twenty flowers as well as petals obtained from them were recorded and wastage percentage was calculated. Wastage is calculated by using following equation and represented as percentage.

$$\text{Wastage (\%)} = \frac{\text{Fresh weight of flower} - \text{fresh weight of petals of flowers}}{\text{Fresh weight of flowers}} \times 100$$

These parameters of harvested fresh mass and useable fresh mass could be helpful as indices for economic estimation of the final products. Before extraction of pigments, fresh petals were dried in shade. The rate of drying, which indicates the percent moisture loss per hour, was calculated using the following equation.

$$\text{Rate of drying (mg hour}^{-1}) = \frac{\text{Initial weight of fresh flower} - \text{Dry weight of flowers}}{\text{Initial weight of fresh flower} \times \text{Time required for drying (hour)}} \times 100$$

Total colour difference is the measurement of colour change estimated as the modulus of the distance from the starting colour value and the true colour coordinates (ΔE*) (Martins and Silva, 2002). In the present experiment the colour difference between two pigments was calculated according to the following equation (Rhim *et al.*, 1999)

$$\Delta E^* = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2}$$

Anthocyanins are water-soluble pigments which impart pink, cyan, blue and purple colours to flowers. For determination of total anthocyanin content, absorbance of light by the pigment solution was read at 520nm wavelength by spectrophotometer and the content was calculated as equivalent to cyanidine-3-glucoside using the formula:

$$\text{Total Anthocyanin content (mgL}^{-1}) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

Where, A represented absorbance at 520nm wavelength, MW (Molecular weight) of cyanidine-3-glucoside represented 449.2 g mol^{-1}, DF means dilution factor, Ɛ denotes molar extinction coefficient of cyanidine-3-glucoside *i.e.* 26900 L mol^{-1} cm^{-1}, 10^3= factor for conversion from g to mg (Lee, 2005; Sarkar *et al*., 2023).

The total carotenoid content of the pigments was estimated by spectrophotometric method as stated by Carvelho *et al.* (2012) using the following formula:

$$\text{Total carotenoid content } (\mu g\ g^{-1}) = \frac{A \times V \times 10^4}{A_{1cm}^{1\%} \times P}$$

Where, A= Absorbance of light at 450nm; V= Total extracted volume in ml; P= Sample weight in gram; $A_{1cm}^{1\%}$= 2592 *i.e.* β-carotene extinction coefficient in petroleum ether.

The experiment was designed according to two-factor Completely Randomized Design with three replications of each of the 18 treatment combinations where the flower species was the first factor and methods of extraction were the second factor. OP STAT software (version 6.8) was used for statistical analysis (Sheoran *et al.*, 1998).

RESULTS AND DISCUSSIONS

Colour of petal

E. indica (S_1) flowers exhibited red colour of darker shade (Red Group 42-A, L*a*b* 46,56,33) compared to the other two species *viz. D. regia* (Orange Red Group N34-B L*a*b* 53,53,37) and *S. campanulata* (Orange Red Group 34-A, L*a*b* 50,53,35).

Fresh weight of flowers, petals and wastage percentage

Significant variation in all the three parameters was noted among the species. *S. campanulata* (S_3) recorded the maximum fresh weight of 20 flowers (80.35g) as well as petals (45.75g), whereas the minimum was noted in *E. indica* (S_1) (11.94g) and (4.82g), respectively (Table 1). It is interesting to note that *E. indica* (S_1) showed minimum fresh weight of flowers yet maximum wastage percentage (59.65%) among the three flowers. This indicates that the recovery of useable parts from *E. indica* flowers is the lowest. Size of petals and petal thickness contributed to this variation.

Dry weight of petals

For comparative study of dry mass content, petals of twenty flowers of each species were subjected to shade drying. Significant variation was evident in the data (Table 1). Notably, maximum dry weight (5.43 g) was recorded in *Delonix regia* petals (S_2) which exceeded *Spathodia* (S_3) in spite of having 40.3% lower fresh weight compared to the later. This suggests a high dry matter content in *Delonix* petals and higher moisture content in *Spathodia* petals.

Rate of drying

The drying rate varied significantly among the three species (Table 1). Maximum rate of drying of 3.69 mg hour^{-1} was recorded in *E. indica* (S_1) followed by *S. campanulata* (S_3) of 1.72 mg hour^{-1} and *D. regia* (S_2) 1.16 mg hour^{-1}. The difference in petal thickness and moisture content may be the cause of this discrepancy. Higher rate of drying can expedite the entire process.

Colour of pigments extracted

The colour of extracted pigments from the three species and by six extraction methods were measured by Hunter Colour Meter and corresponding L* a* b* values are presented in table 2.

The L* value measures the darkness vs lightness of a colour in a range of 0 to100 where lower values represent darker shades. The results indicate that L* values of the pigments varied significantly with species, methods of extraction and interaction of the two factors (Table 2). Among the three species, pigments of *Delonix regia* (S_2) recorded maximum value of L* (22.85) indicating lightest colour followed by *Erythrina indica* (S_1) (L*=22.36). The darkest pigments (L*=20.63) were obtained from *S. campanulata* (S_3). The lightness or darkness of phytopigments is influenced by the anthocyanin and flavonol content and that can vary from species to species and even cultivar to cultivar (Lubel and Brand, 2017). The visible difference in colour of pigments resulted due to difference in methods of extraction was substantiated by the significant difference in their L* values. Lighter colour pigments, with maximum value of L* (25.63), was obtained by the process of soaking and maceration in acidic solution (M_5) and contrastingly, the darkest extracts (L*=19.64) were recovered by soaking and maceration in alkaline solution (M_6). The darkest extract (L*=18.45) was obtained in *S. campanulata* with soaking and maceration in alkaline solution (S_3M_6), while lightest (L*=27.57) from *E. indica* through soaking and maceration in acidic solution (S_1M_5). Torskangerpoll and Andersen (2005) documented that the properties of anthocyanin and its colour expression, which is reflected by its absorbance maxima, changes with its structure and pH. A classical work of Robertson and Robinson (1929) disclosed the colour change of different groups of anthocyanins with varying pH. Pelargonidin chloride became colourless in lower pH (3.0-4.4) while in higher pH (9.8-10.4) the pigment displayed reddish brown colour. Similarly, cyanidin chloride faded to colourless condition from reddish violet or bluish hue within 25 to 45 minutes at lower pH of 3.8-4.4, however, a deeper yellow stable colour was obtained at pH range of 9.8 to 10.4. Pelargonidins and cyanidins are reported to be the major anthocyanins present in the flowers of species under study (Susetyarini *et al.*, 2020; Scogin, 1991; Vankar and Srivastava, 2010; Adje *et al.*, 2008).

Table 1: Fresh weight of flowers, fresh and dry weight of petals, rate of drying and wastage from non-useable parts of flowers of *E. indica*, *D. regia* and *S. campanulate*

Treatment	Fresh weight of 20 flowers (g)	Fresh weight of petals of twenty flowers (g)	Wastage from non-useable parts of flower (%)	Dry weight of petals of twenty flowers (g)	Rate of drying (%moisture loss hour^{-1})
S_1	11.94 ± 0.60	4.82 ± 0.33	59.65 ± 1.046	0.26 ± 0.026	3.69 ± 0.03
S_2	45.77 ± 0.52	27.31 ± 1.39	40.36 ± 2.863	5.50 ± 0.200	1.16 ± 0.01
S_3	80.35 ± 1.32	45.75 ± 1.47	43.02 ± 2.620	4.43 ± 0.256	1.72 ± 0.00

Note: Values are Mean ± SD

Table 2: Colours of *E. indica*, *D. regia* and *S. campanulata* pigments extracted through different methods as represented by L*, a* and b* values

Method of extraction	L*				a*				b*			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
M_1	18.92	20.51	19.80	19.74	1.05	0.96	0.42	0.81	1.52	3.46	0.84	1.94
M_2	22.55	22.25	21.55	22.12	0.08	3.14	0.72	1.31	0.41	4.31	0.55	1.76
M_3	23.07	24.58	18.92	22.18	0.38	1.85	0.73	0.99	0.82	3.94	0.29	1.68
M_4	22.46	24.57	20.46	22.49	0.71	1.17	0.22	0.70	0.56	2.65	2.05	1.75
M_5	27.57	24.70	24.62	25.63	0.22	0.45	0.59	0.42	1.25	0.47	0.53	0.75
M_6	19.56	20.50	18.45	19.51	0.63	0.81	0.31	0.58	1.83	3.84	1.78	2.48
Mean	**22.36**	**22.85**	**20.63**		**0.51**	**1.40**	**0.50**		**1.07**	**3.11**	**1.01**	
Factor	**C.D. at 1%**		**SE(m) ±**		**C.D. at 1%**		**SE(m) ±**		**C.D. at 1%**		**SE(m) ±**	
Species	**0.174**		**0.060**		**0.012**		**0.004**		**0.014**		**0.005**	
Method	**0.245**		**0.085**		**0.017**		**0.006**		**0.019**		**0.007**	
Species x Method	**0.425**		**0.148**		**0.030**		**0.011**		**0.034**		**0.012**	

Ibrahim *et al.* (2011) explained that flavylium cation is the dominant species of anthocyanin present at pH-3 or lower, which offer red, purple to orange shades. When the pH increases to 4-5, colourless carbinol pseudo-bases to yellow retro-chalcones were produced. This is due to thermodynamic as well as kinetic conflict between the hydration interactions of flavylium cation with the proton transfer interactions of hydroxyl groups. This explains the lighter colour of pigments obtained in acidic solution despite of higher anthocyanin contents.

The a* value of a colour denotes its redness *vs.* greenness, where an increasing positive value refer to increasing redness and increasing negative value indicates increasing greenness. As the present study involved red coloured flowers only, the a* values for all the extracts were eventually positive, however, species, methods of extraction and their interaction showed highly significant impact on this index (Table 2). Among the three species *D. regia* (S_2) recorded pigments of +1.40 for a* which was distinctly higher than *E. indica* (S_1) (+0.51) and *S. campanulata* (S_3) (+0.50).

Among the methods of extraction employed, maximum value of a* (+1.31) was obtained with hot water soaking and maceration (M_2) and the lowest value (+0.42) was observed with soaking and maceration in acidic solution (M_5). The data on interaction between the two factors revealed that extraction of *Delonix regia* with hot water soaking and maceration (S_2M_2) yielded more red pigments whereas *Erythrina indica* extract in hot water soaking and maceration (S_1M_2) showed least redness, despite the higher redness of fresh flowers.

The b* value denotes the yellowness *vs.* blueness of a colour, where an increasing positive value refer to increasing yellowness while increasing negative value indicates increasing blueness. All the extracted pigments showed positive value of b* which indicates yellowness (Table 2). Among the species, maximum b* (+3.11) was observed in *D. regia* (S_2). Methods of extraction also significantly affected the b* values. Pigments extracted by soaking and maceration in alkaline solution (M_6) recorded maximum b* (+2.48) whereas the minimum was obtained by soaking and maceration in acidic solution (+0.75). Maximum yellowness (b*= +4.31) was observed in *D. regia* pigment extracted by hot water soaking and maceration (S_2M_2).

Total colour difference (ΔE*)

The ΔE* matrix is presented in table 3. Adekunte *et al.* (2010) analytically classified the colour differences as small difference ($\Delta E^* < 1.5$), distinct ($1.5 < \Delta E^* < 3$) and very distinct ($\Delta E^* > 3$). During this experiment we had studied the Total Colour Difference (ΔE*) of all the colour plates of RHS colour chart and observed that the minimum ΔE* was 1.41 (17D vs 18A; 18C vs 18D). Hence, we considered this value (1.41) as the minimum

ΔE* for a visibly perceivable colour difference. In table 3, the visually non-significant ΔE* (<1.41) are bold faced. Perusal of the data revealed that for cold water extraction (M_1) and alkaline extraction (M_6) resulted in visually similar pigments across the species. Likewise, in S_1, M_2, M_3 and M_4, (the three heating methods employed) resulted in similar colour shade. Even in S_3, the methods M_1, M_3, M_4 and M_6 failed to produce any visual colour difference. On the contrary, the three different heating methods, M_2, M_3 and M_4 resulted in visibly unique colour shades in S_2. Soaking and maceration in acidic solution (M_5) also resulted in pigments with unique colour shade in S_1.

Table 3: Colour difference of *E. indica*, *D. regia* and *S. campanulata* pigments extracted through different methods as represented by Δ E* values

	S_1M_1	S_1M_2	S_1M_3	S_1M_4	S_1M_5	S_1M_6	S_2M_1	S_2M_2	S_2M_3	S_2M_4	S_2M_5	S_2M_6	S_3M_1	S_3M_2	S_3M_3	S_3M_4	S_3M_5	S_3M_6
S_1M_1	**0**																	
S_1M_2	3.9	**0**																
S_1M_3	4.3	**0.7**	**0**															
S_1M_4	3.7	**0.7**	**0.7**	**0**														
S_1M_5	8.7	5.1	4.5	5.2	**0**													
S_1M_6	**0.8**	3.4	3.7	3.2	8	**0**												
S_2M_1	2.5	3.8	3.7	3.5	7.4	1.9	**0**											
S_2M_2	4.8	5	4.5	4.5	6.8	4.4	2.9	**0**										
S_2M_3	6.2	4.4	3.8	4.1	4.3	5.6	4.2	2.7	**0**									
S_2M_4	5.8	3.2	2.5	3	3.4	5.1	4.1	3.5	1.5	**0**								
S_2M_5	5.9	2.2	1.7	2.3	3	5.3	5.2	5.3	3.7	2.3	**0**							
S_2M_6	2.8	4.1	4	3.8	7.6	2.2	**0.4**	3	4.2	4.3	5.4	**0**						
S_3M_1	**1.3**	2.8	3.3	2.7	7.8	**1**	2.8	5	5.9	5.2	4.9	3.1	**0**					
S_3M_2	2.8	**1.2**	1.6	**0.9**	6.1	2.4	3.1	4.5	4.7	3.7	3.2	3.5	1.8	**0**				
S_3M_3	**1.3**	3.7	4.2	3.6	8.7	1.7	3.6	5.7	6.8	6.1	5.8	3.9	**1.1**	2.6	**0**			
S_3M_4	1.8	2.7	2.9	2.5	7.2	**1**	1.6	4.1	4.8	4.3	4.5	1.9	**1.4**	1.9	2.4	**0**		
S_3M_5	5.8	2.1	1.6	2.2	3.1	5.2	5.1	5.1	3.6	2.2	**0.2**	5.3	4.8	3.1	5.7	4.4	**0**	
S_3M_6	**0.9**	4.3	4.7	4.2	9.1	**1.2**	2.7	5.4	6.7	6.2	6.4	2.9	1.6	3.4	1.6	2	6.3	**0**

pH of extracted pigments

The pH of the extracted pigments was recorded (Table 4), since, acid reaction is an important factor guiding selection of pigments for application in food, cosmetics and fabric.

It is noted that species, methods of extraction and their interactions had statistically significant impact on the pH of the pigments. Pigments from *S. campanulata* (S_3) was neutral in reaction with average pH of 7.01, whereas, pigments extracted from *E. indica* (S_1) and *D. regia* (S_2) were of acidic in reaction (pH 5.99 and 5.92, respectively). Maximum pH was recorded with soaking and maceration in alkaline solution (M_6) (10.01) and minimum with soaking and maceration with acidic solution (M_5) (4.03) which is obvious due to use of alkaline and acidic solvents, respectively. However, significant variation in pH is also noted among aqueous extraction methods (M_1, M_2, M_3, M_4) where the samples were subjected to different temperature and application of microwave. It can be further noted that extraction using microwave increases the pH of pigments (6.32) compared to cold water (5.90).

Stability of colour under varying pH condition

The stability of the pigments was judged in low and high pH conditions to estimate the rage of acid reaction in which the pigment retains its original colour. This is pertinent because the pigments are often subjected to varying pH range while being used in food matrix, cosmetics or fabrics. The stability test was done by lowering the pH of the pigments by gradually adding citric acid @ 0.5% and gradual increment of pH was obtained by slowly adding sodium hydroxide @ 0.5%. The pH of the solution was noted as soon as any colour change was observed (Table 4). The results shown that the lower of pH for stability of colour varied significantly with species, method of extraction and their interaction, while the higher range is significantly affected only by the method of extraction and interaction of the factors. Pigments extracted through soaking and maceration in acidic solution (M_5) were stable within the pH range of 2.66 to 10.30 whereas stability of pigments extracted in alkaline solution (M_6) was only between pH 7.40 to 11.82. Irrespective of methods of extraction, pigment of *D. regia* showed higher stability to varying pH compared to other species and the colour was stable within pH 4.27 to 11.66. However, maximum stability was noted in acid extracted *Delonix* pigment (pH 2.48 to 11.51). *S. campanulata* (S_3) pigments on the other hand when extracted in alkaline medium recorded the narrowest range for stability (pH 7.86 to 11.97).

Stability at higher temperature

In the process of dyeing of textile or application in food, the pigments are often subjected to high temperature nearing 100°C. Hence, stability of pigment's colour at higher

temperatures is an essential requirement for their commercial application. The extracted pigments were heated over water bath and the temperature, where any change in colour was visible was recorded and presented in Table 5. Significant variation in temperature stability was noted among the species, methods of application and interactions as well. Pigments extracted from *S. campanulata* (S_3) showed the maximum temperature stability with an average temperature range of 103.66°C. The pigments from *D. regia* (S_2), showed similar temperature stability as of *E. indica* (S_1), with an average temperature range of 92.7°C. Methods of extraction significantly affected the temperature stability. The pigments extracted through boiling in water (M_3) and microwave assisted extraction (M_4) showed higher temperature stability (101.2°C and 103.0°C, respectively) as the extraction procedures involved higher temperature, the contrary was observed in pigments extracted at normal temperature. Pigment of *S. campanulata* extracted through boiling in water (S_3M_4) showed temperature stability up to 108.47°C while pigment from *Delonix regia* flowers extracted in acidic medium (S_2M_5) had minimum temperature stability (up to 82.7°C).

Anthocyanin content

Anthocyanin content in the pigments was distinctly influenced by species, extraction methods and their interaction (Table 5). *S. campanulata* (S_3) and *E. indica* (S_1) were quite similar in anthocyanin concentration (41.06 mg L^{-1} and 40.84 mg L^{-1}, respectively) but pigment of *D. regia* (S_2) flowers was strikingly low in this bio-chemical component (9.73 mg L^{-1}). Glucosides of pelargonidin and cyanidin were reported to be the major anthocyanins in *E. crista-gali* which make up to 85.86% of the pigment (Susetyarini *et al.*, 2020; Scogin, 1991). Methanolic extract of fresh flowers of *D. regia* yielded 101.13 mg kg^{-1} anthocyanin which belongs to three different groups, cyaniding 3-o-glucoside, cyaniding-3-o-rutiniside and pelargonidin-3-o-rutiniside (Vankar and Srivastava, 2010; Adje *et al.*, 2008). Presence of anthocyanin was also reported in the flowers of *Spathodia* (Naglaa *et al.*, 2014).

It was appealing to note that higher anthocyanin recovery was obtained by soaking and maceration in alkaline (M_6) (42.00 mg L^{-1}) and acidic solution (M_5) (30.40 mg L^{-1}) and microwave assisted extraction (M_4) (35.44 mg L^{-1}) whereas the minimum anthocyanin recovery (21.91 mg L^{-1}) was observed in cold water soaking and maceration (M_1). The results are affirmed by the work of Patil *et al.* (2016) reported that the process of extraction of pigments significantly influences their mass transport rate and resultant dye yield in *S. campanulata*.

The species responded differently to the pigment extraction methods for anthocyanin recovery. Alkaline solution (M_6) yielded remarkably higher anthocyanin in all the three species while acidic solution was efficient only in *E. indica* and *D. regia*. Microwave assisted extraction on the other hand, proved to be efficient for anthocyanin extraction from *E. indica* and *S. campanulata* flowers, however, the process was not so effective for *Delonix regia* (S_2). Strikingly, M_2 proved to be the most efficient method for anthocyanin recovery for *S. campanulata* (55.12 mg L^{-1}) but failed to show such effect on the other two species (Scogin, 1991).

Table 4: Effect of species and method of extraction on pH of the pigments, minimum and maximum pH for colour stability of *E. indica, D. regia* and *S. campanulata*

Method of extraction	pH				Minimum pH for colour stability				Maximum pH for colour stability			
	S_1	**S_2**	**S_3**	**Mean**	**S_1**	**S_2**	**S_3**	**Mean**	**S_1**	**S_2**	**S_3**	**Mean**
M_1	5.21	5.09	7.39	5.90	3.78	3.88	5.12	4.26	8.08	11.56	10.87	10.17
M_2	5.32	5.29	6.48	5.70	4.08	4.15	4.15	4.13	11.31	11.46	11.82	11.53
M_3	5.48	5.43	6.71	5.87	4.09	4.09	4.19	4.12	11.85	11.85	11.63	11.78
M_4	5.86	5.75	7.36	6.32	3.98	3.83	3.93	3.91	11.78	11.81	10.79	11.46
M_5	4.02	3.94	4.12	4.03	2.78	2.48	2.72	2.66	11.51	11.51	7.89	10.30
M_6	10.02	10.02	9.98	10.01	7.17	7.17	7.86	7.40	11.69	11.79	11.97	11.82
Mean	**5.99**	**5.92**	**7.01**		**4.31**	**4.27**	**4.66**		**11.04**	**11.66**	**10.83**	
Factor	**C.D. at 1%**		**SE(m) ±**		**C.D. at 1%**		**SE(m) ±**		**C.D. at 1%**		**SE(m) ±**	
Species	**0.008**		**0.003**		**0.010**		**0.003**		**N/A**		**0.321**	
Method	**0.011**		**0.004**		**0.013**		**0.005**		**1.306**		**0.453**	
Species x Method	**0.019**		**0.006**		**0.023**		**0.008**		**2.262**		**0.78**	

Table 5: Effect of species and method of extraction on anthocyanin content, carotenoid content and maximum temperature for colour stability of *E. indica, D. regia* and *S. campanulata* flower pigments

Method of extraction	Anthocyanin content (mg L^{-1})				Carotenoid content (µg g^{-1})				Maximum temperature for colour stability (^{0}C)			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
M_1	37.44	2.59	25.71	21.91	3.08	99.60	6.24	36.31	83.70	87.10	97.77	89.52
M_2	23.08	3.75	55.12	27.32	5.98	91.05	5.98	34.34	89.30	89.80	99.93	93.01
M_3	33.18	6.00	39.39	26.19	23.22	25.59	4.89	17.89	95.77	99.37	108.47	101.2
M_4	50.85	6.92	48.55	35.44	14.59	2.96	2.51	6.68	98.60	102.0	108.4	103.0
M_5	48.27	19.10	23.84	30.40	42.44	87.89	8.10	46.15	100.0	82.70	99.03	93.91
M_6	52.22	20.02	53.75	42.00	37.55	94.46	8.11	46.71	84.80	95.23	108.37	96.13
Mean	40.84	9.73	41.06		21.15	66.93	5.97		92.03	92.70	103.66	
Factor	**C.D. at 1%**		**SE(m) ±**		**C.D. at 1%**		**SE(m) ±**		**C.D. at 1%**		**SE(m) ±**	
Species	0.14		0.05		0.62		0.21		0.23		0.08	
Method	0.21		0.07		0.86		0.30		0.33		0.11	
Species x Method	0.35		0.12		1.49		0.52		0.56		0.19	

Carotenoid content

Carotenoid content in the pigments varied significantly with species, method of extraction and their interaction (Table 5). *D. regia* (S_2), which was lowest in anthocyanin content among the three species, recorded three times higher carotenoid content (66.93 µg g^{-1}) compared to *E. indica* (S_1) (21.15 µg g^{-1}). Very less amount of carotenoid (5.97 µg g^{-1}) was obtained in pigments from *S. campanulata* (S_3). Carotenoid content in ethanolic extract of *D. regia* was recorded as 48.64µg g-1(Kamalambigeswari and Rebecca, 2016). According to Vastard and Goudard (2016) concentration of flavonoid in *S. campanulata* flowers, imparted a reddish hue to the extract, was 17.65 mg g^{-1}.

Methods of pigment extraction also significantly affected the carotenoid content which ranged from 6.68µg g^{-1} to 46.71 µg g^{-1}. Like anthocyanin, recovery of carotenoid pigments was higher in alkaline solution (M_6) and acidic solution (M_5). This might be due to the rupture of cell wall at higher or lower pH and facilitating the extraction process. However, microwave assisted extraction resulted in higher anthocyanin extraction but lower carotenoid content. This may be due to partial degradation of carotenoids in high temperature. Partial degradation of β-carotene and lutein is reported to be as high as -17 to -29% and -3 to -27%, respectively due to high temperature (Abushita *et al.*,1997; D'Evoli *et al.*, 2013). Soaking and maceration in cold (M_1) or hot water (M_2) was effective in extracting carotenoids from *S. campanulata* flowers which yielded 99.60 µg g^{-1} and 91.05 µg $g^{-1}$of flower tissue. However, these methods were not effective for the rest of the species.

CONCLUSION

The present study revealed that, colour, stability and bio-chemical constituent of pigments from flowers is highly influenced by the plant species and extraction condition. Flowers of *Spathodia campanulate* yielded darkest pigments rich in anthocyanins while pigment form *Delonix* flower was more yellowish red, with higher carotenoid content and stability to varying pH. Erythrina flower pigment, though lighter in colour, was more temperature stable. Extraction of floral pigments in alkaline aqueous solution resulted in darker pigment with higher anthocyanin and carotenoid content however, had a narrow range of pH stability. On the other hand, acidic extraction produced unique hues though lighter shades. Micro-wave assisted method proved to be faster and effective way of pigment extraction from anthocyanin rich flowers.

ACKNOWLEDGEMENT

The authors humbly acknowledge the support rendered by the Department of Floriculture, Medicinal and Aromatic Plants and Central Quality Analysis Laboratory, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal. The authors also acknowledge the Department of Higher Education Science and Technology and Bio-technology, Govt. of West Bengal for funding the research.

REFERENCES

Abushita, A. A., Hebshi, E. A., Daood, H. G. and Biacs, P. A. 1997. Determination of antioxidant vitamins in tomatoes. *Food Chem.*, **60**: 207-12.

Adekunte, A.O., Tiwari, B.K., Cullen, P.J., Scannell, A.G.M. and O'donnell, C.P. 2010. Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chem.*, **3**: 500-507.

Adje, F., Lozano, Y. F., Meudec, E., Lozano, P., Adima, A., N'zi, G. A. and Gaydou, E. M. 2008. Anthocyanin characterization of pilot plant water extracts of *Delonix regia* flowers. *Molecules*, **13**: 1238-45.

Carvalho, L. M. J., Gomes, P. B., Godoy, R. L. O., Pacheco, S., Monte, P. H. F., Carvelho, J. L. V.,

Nutti, M. R., Neves, A. C. L., Vieira, A. C. R. A. and Ramos, S. R. R. 2012. Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. *Food Res. Int.*, **47**: 337–40.

D'Evoli, L., Lombardi-Boccia, G. and Lucarini, M. 2013. Influence of Heat Treatments on Carotenoid Content of Cherry Tomatoes. *Foods*, **2**: 352-63.

Devi, Y. P. 2019. Traditional Natural Dyes Used for Dyeing Fibre and Fabrics of Manipur. *Int. J. Res. Review*, **6**: ISSN: 2349-9788.

Divya, K. R. and Manonmani, K. 2014. Utilization of flower dyes on silk and cotton using mordant combinations. *Elixir Int. J.*, **6**: 20764-20766.

Ibrahim, U. K., Muhammad, I. I. and Salleh, R. M. 2011. The Effect of pH on Color Behavior of *Brassica oleracea* anthocyanin. *J. Appl. Sci.*, **11**: 2406-10.

Kamalambigeswari, R. and Rebecca, J. L. 2016. Extraction of Major Carotenoids from Flower Petals. *Int. J. Pharm. Sci. Rev. Res*, **39**: 37-39.

Lee, J. 2005. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *J. AOAC Int.*, **88**: 1269-78.

Lokesh, P. and Swamy, M. K. 2013. Extraction of natural dyes from *Spathodea campanulata* and its application on silk fabrics and cotton. *Pelagia Res. Lib. Der Chemica Sinica*, **4**:111-115.

Lubel, J. D. and Brand, M. H. 2017. Flower Color, Color Stability, and Flower Longevity in Red-flowered Elepidote Rhododendrons. *Hort Tech.*, **27**: 607-10.

Martins, R. C., and Silva, C. L. M. 2002. Modelling colour and chlorophyll losses of frozen green beans (*Phaseolus vulgaris*, L.). *Int. J. Refrig.*, **7**: 966-74.

Naglaa, G. S., Hanaa, H. E. and Soheir, M. 2014. Bioactivity and composition of the flowers of *Spathodea campanulata* p. *Beauv. World J. Pharm. Res.*, **3**: 213-30.

Parthasarathi, B. and Lokesh, P. 2015. A case study of natural dye extraction and phytochemical screening using the flower *Spathodea campanulata*. *Int. J. Adv. Phar., Biol. Chem.*, **4**: ISSN: 2277– 4688.

Patil, P. D., Rao, C. R., Wasil, A. I. and Anekar, S. V. 2016. *Spathodea campanulata* Beauv. Flower dye extraction: Mass transfer enhancement through process optimization. *Indian J. Chem. Tech.*, **23**: 303-307.

Rhim, J. W., Y. Wu, C. L. Weller, and M. Schnepf. 1991. Physical characteristics of a composite film of soy protein isolate and propyleneglycol alginate. *J. Food Sci.*, **1**: 149-52.

Robertson, A. and Robinson, R. 1929. Note on the characterisation of the anthocyanins and anthocyanidins by means of their colour reactions in alkaline solutions. *Biochem. J.*, **23:** 35.

Rymbai, H., Sharma, R. R. and Srivastav, M. 2011. Biocolorants and its implications in Health and Food Industry - A Review. *Int. J. Pharm. Tech. Res.*, **3**: 2228-44.

Sarkar, D., Khan, A. M., Maitra, S. and Paul, P. K. 2023. Standardization of Bio-pigment Extraction Techniques from Yellow Flowering Landscape Ornamentals. *Biological Forum – An Int. J.*, **15**: 455-61.

Scogin, R. 1991. Anthocyanins of the genus *Erythrina* (Fabaceae). *Biochem. Syst. Ecol.*, **19**: 329-332, ISSN 0305-1978

Sheoran, O.P., Tonk, D.S., Kaushik, L.S., Hasija, R.C. and Pannu, R.S. 1998. Statistical Software Package for Agricultural Research Workers. Recent Advances in information theory, Statistics & Computer Applications by D.S. Hooda & R.C. Hasija Department of Mathematics Statistics, CCS HAU, Hisar: 139-43.

Singh, R. 2017. Sources of Natural Dye - A Critical Review. *Int. J Engr. Sci. Math.*, **6**: ISSN: 2320-0294

Singh, S. V. and Purohit, M. C. 2012. Applications of Eco-Friendly Natural Dye on Wool Fibers Using Combination of Natural and Chemical Mordants. *Universal J. Environ. Res. Tech.*, **2**: 48-55.

Siva, R. 2007. Status of natural dyes and dye-yielding plants in India. *Current Sci.*, **7**: 916-925.

Susetyarini, E., Wahyuni, S., Kharoir, I., Husamah, and Setyawan, D. 2020. Influence of Erythrina crista-galli L. Extract Natural Dye in Plant Histology Staining. *Int. Conf. Life Sci. Tech.*, **2231**: 040027.

Torskangerpoll, K. and Andersen, Ø. M. 2005. Colour stability of anthocyanin in aqueous solutions at various pH values. *Food Chem.*, **89**: 427-40.

Vadivelan, V., and Kumar, K. V. 2005. Equilibrium, kinetics, mechanism, and process design for the sorption of methylene blue onto rice husk. *J. Colloid Interface Sci.*, **286**: 90-100.

Vankar, P. S. and Shanker, R. 2009. Eco-friendly pretreatment of silk fabric for dyeing with *Delonix regia* extract. *Coloration Tech.*, **125**: 155-60.

Vankar, P. S. and Srivastava, J. 2010. Evaluation of Anthocyanin Content in Red and Blue Flowers. *Int. J. Food Engr.*, **6**.

Vastrad, J. V. and Goudar, G. 2016. Evalution of Phenolic Compounds and Development of Chromatographic Profiles in *Spathodea campanulata* Inflorescence by HPTLC. *Asian J. Chem.*, **28**: 497-500.

Verma, S. and Gupta, G. 2017. Natural dyes and its applications: A brief review. *Int. J. Res. Anal. Rev.*, **4**:ISSN 2348 –1269.

Yaneva, Z. L. and Georgieva, N. V. 2012. Insights into Congo Red Adsorption on Agro-Industrial Materials Spectral, Equilibrium, Kinetic, Thermodynamic, Dynamic and Desorption Studies. A Review. *Int. Rev. Chem. Engr.*, **4**: 127-46.